AD_____

GRANT NO: DAMD17-94-J-4247

TITLE: Genetic Analysis of Human Breast Cancer

PRINCIPAL INVESTIGATOR(S):   Dr. Michael H. Wigler
                             Dr. Nikolia Lisitsyn

CONTRACTING ORGANIZATION: Cold Spring Harbor Laboratory
                            Cold Spring Harbor, New York   11724

REPORT DATE: August 14, 1995

TYPE OF REPORT: Annual
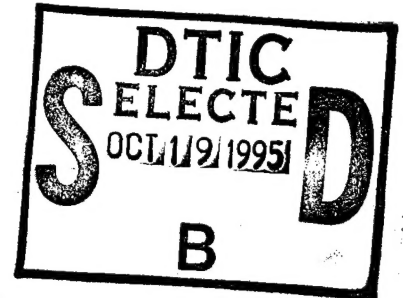
PREPARED FOR: Commander
                   U.S. Army Medical Research and Materiel Command
                   Fort Detrick, Maryland   21702-5012

DISTRIBUTION STATEMENT: Approved for public release;
                          distribution unlimited

The views, opinions and/or findings contained in this report are those
of the author(s) and should not be construed as an official Department
of the Army position, policy or decision unless so designated by other
documentation.

REPORT DOCUMENTATION PAGE

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503.

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE Aug. 14, 1995	3. REPORT TYPE AND DATES COVERED Annual July 15, 1994 – July 14, 1995

4. TITLE AND SUBTITLE	5. FUNDING NUMBERS
Genetic Analysis of Human Breast Cancer	DAMD17-94-J-4247

6. AUTHOR(S)

Dr. Michael H. Wigler/Dr. Nikolia Lisitsyn

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)	8. PERFORMING ORGANIZATION REPORT NUMBER
Cold Spring Harbor Laboratory Cold Spring Harbor, New York 11724	705100

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)	10. SPONSORING/MONITORING AGENCY REPORT NUMBER
U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012	

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION/AVAILABILITY STATEMENT	12b. DISTRIBUTION CODE
Approved for public release, distibution unlimited	

13. ABSTRACT (Maximum 200 words)

Cancer is a disorder brought upon by the accumulation of specific mutations in the cancerous cells. Our understanding of the disease, and potentially its diagnosis and therapeutic treatment, is enhanced by defining the genetic lesions that cause it. Representational difference analysis, or RDA, was developed to do just this. RDA is a DNA subtraction methodology that finds sequences present in one DNA population, the tester, that is absent or reduced in a second, the driver (1, 2). RDA has been used to discover sequences lost or amplified in the genomic DNA of the cancerous cells (3). Genetic loss and gene amplification are hallmarks of tumor suppressor genes and oncogenes, respectively. We have been applying RDA to the discovery of sequences lost in breast cancer. At least three loci undergoing loss in breast cancer have been identified. The transcriptional potential of these loci is being explored. Characterization of many other RDA probes is in progress. We expect that the continued execution of our stated plan will accomplish our stated goal, the identification of tumor suppressor genes that are commonly involved in breast cancer.

14. SUBJECT TERMS	15. NUMBER OF PAGES 7
Oncogenes, Tumor suppressors, DNA,difference analysis, Genetic Lesions	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)
Prescribed by ANSI Std. Z39-18
298-102

GENERAL INSTRUCTIONS FOR COMPLETING SF 298

The Report Documentation Page (RDP) is used in announcing and cataloging reports. It is important that this information be consistent with the rest of the report, particularly the cover and title page. Instructions for filling in each block of the form follow. It is important to *stay within the lines* to meet *optical scanning requirements*.

Block 1. Agency Use Only *(Leave blank)*.

Block 2. Report Date. Full publication date including day, month, and year, if available (e.g. 1 Jan 88). Must cite at least the year.

Block 3. Type of Report and Dates Covered. State whether report is interim, final, etc. If applicable, enter inclusive report dates (e.g. 10 Jun 87 - 30 Jun 88).

Block 4. Title and Subtitle. A title is taken from the part of the report that provides the most meaningful and complete information. When a report is prepared in more than one volume, repeat the primary title, add volume number, and include subtitle for the specific volume. On classified documents enter the title classification in parentheses.

Block 5. Funding Numbers. To include contract and grant numbers; may include program element number(s), project number(s), task number(s), and work unit number(s). Use the following labels:

C	- Contract	PR	- Project
G	- Grant	TA	- Task
PE	- Program Element	WU	- Work Unit Accession No.

Block 6. Author(s). Name(s) of person(s) responsible for writing the report, performing the research, or credited with the content of the report. If editor or compiler, this should follow the name(s).

Block 7. Performing Organization Name(s) and Address(es). Self-explanatory.

Block 8. Performing Organization Report Number. Enter the unique alphanumeric report number(s) assigned by the organization performing the report.

Block 9. Sponsoring/Monitoring Agency Name(s) and Address(es). Self-explanatory.

Block 10. Sponsoring/Monitoring Agency Report Number. *(If known)*

Block 11. Supplementary Notes. Enter information not included elsewhere such as: Prepared in cooperation with...; Trans. of...; To be published in.... When a report is revised, include a statement whether the new report supersedes or supplements the older report.

Block 12a. Distribution/Availability Statement. Denotes public availability or limitations. Cite any availability to the public. Enter additional limitations or special markings in all capitals (e.g. NOFORN, REL, ITAR).

DOD - See DoDD 5230.24, "Distribution Statements on Technical Documents."
DOE - See authorities.
NASA - See Handbook NHB 2200.2.
NTIS - Leave blank.

Block 12b. Distribution Code.

DOD - Leave blank.
DOE - Enter DOE distribution categories from the Standard Distribution for Unclassified Scientific and Technical Reports.
NASA - Leave blank.
NTIS - Leave blank.

Block 13. Abstract. Include a brief *(Maximum 200 words)* factual summary of the most significant information contained in the report.

Block 14. Subject Terms. Keywords or phrases identifying major subjects in the report.

Block 15. Number of Pages. Enter the total number of pages.

Block 16. Price Code. Enter appropriate price code *(NTIS only)*.

Blocks 17. - 19. Security Classifications. Self-explanatory. Enter U.S. Security Classification in accordance with U.S. Security Regulations (i.e., UNCLASSIFIED). If form contains classified information, stamp classification on the top and bottom of the page.

Block 20. Limitation of Abstract. This block must be completed to assign a limitation to the abstract. Enter either UL (unlimited) or SAR (same as report). An entry in this block is necessary if the abstract is to be limited. If blank, the abstract is assumed to be unlimited.

FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the US Army.

MW Where copyrighted material is quoted, permission has been obtained to use such material.

MW Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

MW Citations of commercial organizations and trade names in this report do not constitute an official Department of Army endorsement or approval of the products or services of these organizations.

MW In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

MW For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

MW In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

MW In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

MW In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

Michael H M ϕ̸le₁   Ay 12 1995
_____   _____
PI - Signature   Date

3

TABLE OF CONTENTS

Page Number

INTRODUCTION

Cancer is a disorder brought upon by the accumulation of specific mutations in the cancerous cells. Our understanding of the disease, and potentially its diagnosis and therapeutic treatment, is enhanced by defining the genetic lesions that cause it. Representational difference analysis, or RDA, was developed to do just this. RDA is a DNA subtraction methodology that finds sequences present in one DNA population, the tester, that is absent or reduced in a second, the driver (1, 2). RDA has been used to discover sequences lost or amplified in the genomic DNA of the cancerous cells (3). Genetic loss and gene amplification are hallmarks of tumor suppressor genes and oncogenes, respectively. We have been applying RDA to the discovery of sequences lost in breast cancer.

RESULTS

The application of RDA to cancer requires the availability of matching tumor and normal DNA from the same individual, as otherwise the cloning of DNA polymorphisms results. The vast majority of available tumor material is not provided with accompanying normal cell samples. However, all tumors contain normal stroma. Since many tumors are aneuploid, we have chosen to apply RDA to tumor biopsies that have been sorted by flow cytometry into aneuploid (tumor) and diploid (normal) nuclei. Our studies have confirmed the utility of samples prepared in this way (3).

To date a total of 250 human breast cancer biopsy samples have been obtained from collaborating hospitals. These include 123 from Sloan-Kettering Memorial Hospital, 58 from the Cooperative Human Tissue Network, 65 from North Shore University Hospital, and 4 from Nassau County Medical Center. DNA content analysis by flow cytometric techniques have been performed on 198 of these samples. Of the samples analyzed a total of 51 have been sorted into diploid and aneuploid fractions. From these sorted fractions DNA has been prepared for RDA. An additional 17 samples have been identified for sorting from the initial 198 analyzed samples.

Seven pairs of normal and tumor DNAs have been analyzed by RDA using DNA samples isolated from aneuploid nuclei, fractionated by fluorescence-activated cell sorter from breast cancer biopsies. Many candidate probes have been isolated. Two probes have been characterized to date. One detected loss of heterozygosity of a polymorphic marker. Another nonpolymorphic probe presumably detected homozygous loss.

The last probe has been used to screen a P1 phage human genomic library. The ends of P1 clones have been cloned and their sequences as well as

the sequence of the original probe have been used for synthesis of three pairs of PCR primers. No additional homozygous losses have been detected with this probe in 150 tumor DNAs isolated from our collection of cell lines.

The P1 clones described above have been located by flourescent in situ hybridization to the long arm of chromosome 22. This region has been shown to be frequently deleted in breast tumors. In collaboration with Human Genome Center for chromosome 22 (The Children's Hospital of Philadelphia) these probes have been placed on a YAC contig in a region 22q11.12 positioned three megabases apart from the centromere, and several megabases apart from the site of constitutional reciprocal translocation t(11q;22q) found to be associated with increased risk of breast cancer.

In addition to these studies, we have identified loci that undergo deletion in colon and kidney cancers. Probes from these loci detect deletion in one breast cancer cell line. In particular two probes located on chromosomes 3 (band p21) and 20 (band p11) generated in studies of DNA losses in colorectal tumors, have been found to be simultaneously missing in breast cancer cell line MDA-MB-436, indicating that potential tumor supressor genes, which are encoded in these regions, are involved in different pathways. The original two probes have been used for screening YAC libraries and several additional sequences from the same genomic region have been subcloned from each YAC, using new subtraction technology which we developed for this purpose.

Frequent homozygous losses of these sequences have been detected by the polymerase chain reaction in a collection of DNAs isolated from >200 cancer cell lines of different origin and the regions of common loss on chromosomes 3 and 20 have been identified. The probes from these regions have been found to be homozygously lost with remarkable frequency (14.9% and 7% correspondingly) in cell lines established from tumors of the gastrointestinal tract (stomach, duodenum, colon, rectum). We have focused our efforts on positional cloning of the candidate genes from these loci.

To make a physical map of the chromosome 3 region, four P1 and nine cosmid clones have been isolated. We applied an exon-trapping system (4) to the clones and 12 exon candidates were identified. Further analysis revealed that two of them are evolutionarily conserved and that three are expressed in brain and kidney. Full length cDNA is being cloned. As for chromosome 20, two P1 clones have been isolated and were analyzed by the exon-trapping system. Three exon candidates have been isolated. Screening of cDNA liraries is being carried out.

CONCLUSIONS

RDA is an effective way to identify regions of genetic change in cancers, and flow cytometry is an effective way to obtain material for analysis. At least three loci undergoing loss in breast cancer have been identified. The transcriptional potential of these loci is being explored. Characterization of many other RDA probes is in progress. We expect that the continued execution of our stated plan will accomplish our stated goal, the identification of tumor suppressor genes that are commonly involved in breast cancer.

REFERENCES

1. Lisitsyn, N. , Lisitsina, N. and Wigler, M. (1993) Cloning the differences between two complex genomes. *Science* 259, 946-951.

2. Lisitsyn, N., and Wigler, M. (1995) Applications of representational difference analysis to detection of genetic lesions in cancer. *Methods in Enzyomology*, in press.

3. Lisitsyn, N. A., Lisitsina, N. M., Dalbagni, G., Barker, P., Sanchez, C. A., Gnarra, J., Linehan, W. M., Reid, B.J. and Wigler, M.H. (1995) Comparative genomic analysis of tumors: Detection of DNA losses and amplification.*Proc. Natl Acad Sci. USA* 92, 151-155.

4. Buckler, A.J., Chang, D.D., Graw, S.L., Brook, J.D., Haber, D.A., Sharp, P.A., and Housman, D.E. (1991) Exon amplification: A strategy to isolate mammalian genes based on RNA splicing. *Proc. Natl. Acad. Sci. USA* 88, 4005-4009.

PERSONNEL

Drs. Nikolai Lisitsyn, Hao Peng Xu, Peter Barker, Robert Lucito, Masaaki Hamaguchi, Linda Rodgers, Wen Wei.